of loop lengths across a large range of LacI concentrations, overwhelms the effect of HU. Indeed, this last possibility may be significant, since the formation of a 400 bp loop with wild-type spacing and Lac loop operators, O_1 and O_2, exhibits a narrower range of looping as a function of LacI concentration with a maximum of 50%.

379-Pos Board B159
Using Contrast Variation with SAXS to Visualize DNA Dissociation from Nucleosome Core Particles
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Nucleosome core particles (NCP) make up the fundamental repeating structures that package and regulate access to eukaryotic chromatin. High resolution crystal structures deliver detailed snapshots of the NCP in its most stable conformations, which consists of 147 base-pairs of DNA tightly wrapped around an octamer of histone proteins. However, the intermediate states of the NCP, as the DNA unwinds from the histone core, are biologically interesting and can be challenging to characterize. To this end, we applied small angle x-ray scattering (SAXS) - a powerful technique for resolving global properties of macromolecules in solution. In order to resolve the ambiguity that arises from the different scattering properties between proteins and nucleic acids within complexes, we applied a contrast variation approach to effectively probe the DNA component of the NCP during salt-induced disassembly. We measured the equilibrium response of NCPs in increasing concentrations of NaCl and modelled the DNA conformations as it unwraps from the NCP. These results highlight the strength of SAXS with contrast variation as a platform for studying protein-nucleic acid complexes.

380-Pos Board B160
Effects of Histone Variants MacroH2A and H2A.Z on Nucleosome Dynamics
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The Nucleosome Core Particle (NCP) forms the fundamental unit of DNA packaging in chromatin fibers. Historically, the NCP has been considered static and in a closed conformation. However, recent evidence suggests that it may have dynamical properties that play a key role in its structural stability and transcriptional accessibility. Modifications to the NCP via histone variant substitutions can alter the stability of the complex. For example, replacement of the canonical histone H2A by variants macroH2A or H2A.Z has been linked to transcriptional repression, the mechanisms of which are not well understood. Here, we have used all-atom molecular dynamics simulations to provide insight into the mechanism by which variants macroH2A and H2A.Z stabilize the NCP. Our results show that dynamics of variant nucleosomes deviate from the canonical NCP primarily at the local level on the hundreds of nanoseconds timescale. Inclusion of variants alters the dynamics of the entry and exit segments of the DNA in the form of tighter DNA winding. Simulations also suggest that the presence of variants results in tighter dimer compaction. Furthermore, mutations to the dimer interface elucidate allosteric pathways to key locations of the complex.

381-Pos Board B161
Histone Phosphorylation Combined with Acetylation Dramatically Increase Nucleosome Accessibility
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Histone post translational modifications (PTMs) regulate DNA transcription, replication and repair. A number of histone PTMs have been identified within the DNA entry-exit region of the nucleosome including phosphorylation of H3 at tyrosine 41 (H3Y41ph) and threonine 45 (H3T45ph). Each of these modifications are implicated to occur with H3K56ac, which is known to increase DNA unwrapping and accessibility. However, the influence of H3Y41ph and H3T45ph on nucleosome dynamics and stability with and without H3K56ac remains undetermined. Micrococcal nuclease digestion, small angle x-ray scattering and fluorescence resonance energy transfer (FRET) measurements reveal that each phosphorylation mimics H3Y41E and H3T45E, and the exact modification, H3Y41ph that was prepared by sequential native ligation, increase nucleosome unwrapping and enhance DNA accessibility to protein binding by three fold. Combinations of either phosphorylation site with H3K56ac increased DNA accessibility multiplicatively, while the combination of the phosphorylation sites did not increase accessibility beyond single phosphorylations. These results show that H3Y41 and H3T45 phosphorylation increase nucleosome unwrapping and accessibility similarly to H3K56 acetylation, and that combinations of PTMs can function multiplicatively to increase DNA accessibility by more than an order of magnitude.

382-Pos Board B162
Glucocorticoid Receptor-DNA Dissociation Kinetics Measured in vitro Reveal Exchange on the Second Timescale
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The glucocorticoid receptor (GR) is a member of the steroid receptor family of ligand-activated transcription factors. Recent live cell imaging studies have revealed that GR interactions with chromatin are highly dynamic, with receptor residence times on the order of seconds. These findings were surprising since early in vitro studies suggested that GR-DNA interactions were more static, with receptor-DNA exchange occurring on the order of minutes to hours. However, these latter analyses were carried out without the modern-day advantages of recombinant receptor expression, established purification protocols or rigorous characterization methods. Here we examine the DNA dissociation kinetics of full-length human GR, using highly purified and homogenous receptor preparations. We find that in vitro dissociation kinetics are not slow as previously reported, but are quite fast, with calculated receptor-DNA residence times of seconds to tens of seconds. These findings are observed at both isolated response elements and the multisite mouse mammary tumor virus (MMTV) promoter used in live cell imaging. However, we also find that dissociation kinetics are identical for all response elements and are biphasic in character. These results indicate that differences in GR affinity toward these sequences are not due to differences in off-rate but on-rate, and further suggest that GR exists in multiple and interconverting states when bound to DNA. Since we previously established that GR-DNA binding affinity is a primary determinant of transcriptional activity at these sequences, the current findings implicate DNA sequence in controlling the extent of receptor interconversion and hence extent of transcriptional activation.

383-Pos Board B163
Global Characterization of Transcription Factor Localization and Partitioning in Escherichia Coli
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Despite the lack of cytoskeletal molecular motors and membrane-bound organelles, the bacterial cell exhibits surprising complexity in subcellular structure and organization. To isolate the localization behavior of bacterial proteins at the genome-scale, we combine high-throughput time-lapse fluorescence microscopy and automated image analysis to capture the cell-cycle localization dynamics of nearly every protein in E. coli that exhibits a non-diffuse localization pattern. We capture hundreds of complete cell cycles for each protein, which facilitates quantitative analysis of cell-cycle dynamics and cell-to-cell variation. We exploit this unique data set to characterize the localization behavior of a subset of proteins that exhibit large variability in localization patterns: DNA-binding transcription factors. We find that many transcription factors form well localized punctate foci with significant complexity in positioning, both spatially and temporally. Principal components analysis reveals that these proteins occupy diverse yet overlapping localization groups, many of which are cell-cycle dependent. Finally, we find numerous examples of non-trivial partitioning of transcription factors at cell division, including proteins that appear to be preferentially partitioned as a function of cell age. This complex localization behavior may be evidence of a new global model for the function of transcription factors in bacterial cells.

384-Pos Board B164
Unraveling the Structural, Dynamic, Thermodynamic, and Kinetic Heterogeneity in DNA Site Recognition by Structurally Homologous ETS Transcription Factors
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Despite increasing awareness of their biological importance, mechanisms of DNA site discrimination by structurally homologous and functionally non-redundant transcription factors remain poorly defined concepts. Members of